Role of Marker Lesion when Applying Intravesical Instillations of IL-2 for Non-muscle-invasive Bladder Cancer Comparison of the Therapeutic Effects in Two Pilot Studies

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Abstract. *Aim: Comparison of the therapeutic effect of treatment of non-muscle invasive bladder carcinoma (NMIBC) after intravesical Interleukin-2 (IL-2) instillations in the presence and absence of a marker tumour. Materials and Methods: Two pilot studies were performed in patients with NMIBC. The first study (10 patients) was performed in Krakow (Poland), the second (26 patients) in Vilnius (Lithuania). In Krakow the tumours were treated with incomplete transurethral resection (TUR) leaving a marker tumour of 0.5-1.0-cm followed by IL-2 instillations (3×10^6 IU IL-2) on five consecutive days. In Vilnius the tumours were treated with complete TUR, followed by IL-2 instillations (9×10^6 IU IL-2) on five consecutive days. Results: During 30 months follow-up, the recurrence-free survival was 5/10 (50%) and 6/26 (23%) after incomplete and complete TUR, respectively. So, the ratio of the recurrence-free survival after incomplete/complete TUR of 50/23=2.2. The median of the recurrence-free survival is >20.5 months and 7 months after incomplete and complete TUR, respectively. So, this ratio was >20.5/7= >2.9. The hazard ratio which combines both the chance of the disease recurrence and its timing for both censored and uncensored cases was 0.53, again confirming the better outcome after incomplete TUR. Conclusion: A possible explanation for the better therapeutic effects after incomplete TUR compared with complete TUR is that the marker tumour has tumour-associated antigens (TAA) that could lead to an immune reaction that is stimulated by local application of IL-2. After complete TUR, no TAA are available to initiate and to stimulate an immune reaction; consequently, local IL-2 therapy is less effective after complete TUR. The results of these two pilot studies have led to the recent start of a randomised prospective clinical trial in which therapeutic effects of local IL-2 therapy after complete and incomplete TUR are compared.*

Non-muscle invasive bladder carcinoma (NMIBC) should be treated with complete transurethral resection (TUR), in order to obtain optimal staging and to reduce the chance of tumour recurrence. However, recurrence rates at first follow-up cystoscopy at three months varied between 7.4-45.8%, which is a sign that the technique for TUR needs improvement (1). The indication to start adjuvant chemo- or immunotherapy is based on the results of the pathological and clinical findings, because after evaluation of several randomized EORTC-GU-group trials, patients can be classified into different risk group categories for recurrence and progression, if there is no invasion into the

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muscle layer (2). Adjuvant chemo- and immunotherapy cause side-effects, especially when given for a longer period, and there are no prognostic factors identifying which patients need either of the two treatments and, more importantly, there are no predictive tools to identify those patients in whom disease will progress in spite of or during adjuvant instillation. Improvements in this field of genito-urinary cancer are, thus, still needed.

In order to avoid exposing many patients in a randomized phase III trial to new intravesical agents, the EORTC-GU-group developed the concept of the marker tumour (3-5): during TUR, an untouched marker lesion of 0.5-1.0 cm is deliberately left behind and two months after a course of intravesical instillations, the effect of the investigational agent on the marker tumour is evaluated by cystoscopy under anaesthesia and resection of the tumour or place where the marker lesion was located is carried out; the pathological report has to indicate if there is still tumour or not. If the marker tumour is not present anymore, then it is regarded to be due to the therapeutic activity of the instilled investigational drug. If the marker has not disappeared completely, then the instillation therapy is regarded to be ineffective. The evaluation of response to a marker tumour has been proven to be a safe method to test new drugs (3-5). At the Fourth International Bladder Consensus Conference, it was recommended that marker tumour phase-II studies should be adopted as a standard clinical trial practice for the evaluation of new agents to treat NMIBC before embarking on large phase-III studies (3).

Interleukin-2 (IL-2) has been used for cancer treatment with local (intratumoural or peritumoural) application (6, 7). Therapeutic effects were obtained in tumour models in mice and rabbits (6, 8), and in veterinary (9, 10) and human (11, 12) cancer patients. It was, thus, of interest to treat patients with NMIBC with incomplete TUR, leaving a marker tumour, followed by IL-2 instillation. Applying IL-2 instillation in patients with NMIBC was interesting, because urinary IL-2 levels were increased in patients showing a positive response to immunotherapeutic instillations (13-15).

In Krakow, patients were treated in a pilot study with incomplete TUR followed by IL-2 instillations (11). To test the reproducibility of the data, an identical trial was planned in Vilnius (16, 17). However, the urologists in Vilnius regarded application of the marker tumour concept to be unethical, as they thought that this might harm the patients, due to tumour progression. Hence, they performed complete TUR. This enabled us to compare the effects of intravesical IL-2 therapy in NMIBC in the presence and the absence of a marker tumour.

The therapeutic influence of incomplete and complete TUR was expressed as: the ratio of the % of cases that did not develop a recurrence during the follow-up period after incomplete and complete TUR; the ratio of the median time until tumour recurrence after incomplete and complete TUR; the Kaplan Meier plot combining number and timing of recurrences after incomplete and complete TUR.

Table I. *Characteristics of patients in Krakow*.*

Patient number	Age (Years)	Gender	Stage/ grade	Number of preoperative tumours	Recurrence rate/ year
1	54	M	pT1G1	3	3.3
2	34	M	pT1G1	6	1.1
3	67	F	pT1G1	2	2.5
4	62	F	pT1G1-2	11	1.0
5	47	M	pT1G1	3	1.2
6	43	F	pT1G1	3	2.0
7	53	F	pT1G1	2	6.0
8	71	M	pT1G2	3	-
9	63	M	pT1G1	2	-
10	69	M	pT1G1	3	0.4

*Clinical study performed in Krakow (11). These patients received incomplete TUR and IL-2 instillations.

If the ratio after incomplete/complete TUR is 1, then both therapies exert an equal therapeutic effect; if the ratio is 2, then the most effective therapy is twice as effective as the other therapy. *Etc*.

The Krakow study

Krakow: Materials, Methods and Results

In the first pilot study ten patients who had stage T1, grades 1 or 2 NMIBC were treated with incomplete TUR, leaving a marker tumour. The patients gave their informed consent and the Ethics Committee of the Jagellonian University Medical College in Krakow, Poland, approved the protocol. Patients' characteristics are shown in Table I. At TUR, a tumour of 0.5-1.0 cm was left as a marker tumour. Cancer was histologically-proven using the removed tumour tissue. Two days after TUR, when the diagnosis was histologically-confirmed, 3×10^6 units IL-2 in 50 ml saline plus 0.1% human serum albumin (HSA) were instilled in the bladder through a catheter. The IL-2 solution remained in the bladder for two hours. IL-2 was instilled on five consecutive days. The effect of IL-2 treatment on the marker tumour was evaluated by cystoscopy and repeat biopsy of the marker site two months after treatment. Any marker tumour tissue present after two months was resected at that time. Thereafter, during follow-up visits, tumour recurrence was assessed with cystoscopy and biopsy. In case of tumour recurrence, the patient was removed from the study. The IL-2 was produced by Chiron/Novartis. Data of this study have been published by Den Otter *et al*. in 1998 (11). The results are summarized in Table II. Following treatment complete response (CR; no tumour or tumour cells were detectable anymore) was demonstrated in 5/10 (50%) patients during the whole follow-up period of 30-54 months. The median recurrence-free interval was > 20.5 months (>30+11)/2). The findings for patients 1 and 2 in Tables I and

Table II. *Time (months) between treatment and recurrence after incomplete TUR and local IL-2*.*

Patient number	Months between treatment and recurrence	No recurrence during whole follow-up period (Months)
1		>54
2		>54
3		>30
4	2	
5	2	
6	11	
7		>36
8	5	
9		>30
10	2	

*Clinical study performed in Krakow (11).

II are interesting, since these patients had bladder carcinoma for 7 and 11 years and were treated with 23 and 12 TURs; after incomplete TUR and IL-2 instillations, both patients were tumour-free during the whole follow-up period of 54 months.

The Vilnius study

Vilnius: Materials, Methods and Results

In the second pilot study, 26 consecutive patients with stage Ta-T1 G1, G2, or G3 NMIBC were treated with complete TUR and intravesical IL-2 instillations. All patients gave their written informed consent. This protocol was approved by the Lithuanian Bioethics Committee and State Medicines Control Agency of Lithuania (trial registration: EudraCT: 2004-002821- 30).

Patients' characteristics are shown in Table III. Two days postoperatively, following pathological confirmation of NMIBC, $9X10^6$ units IL-2 (Chiron/Novartis) in 50 ml saline were instilled in the bladder through a catheter. The IL-2 solution remained in the bladder for two hours. IL-2 was instilled on five consecutive days. The effect of the TUR plus IL-2 therapy was evaluated during the regular follow-up visits (cystoscopy and cytology) until tumour recurrence. If tumour recurrence was demonstrated, the patient was removed from this study. Preliminary data of this study have been published by our group in 2010 and 2011 (16, 17).

The results of the Vilnius study are shown in Table IV. A total of 2/26 of these patients (8 %) did not experience a recurrence within the follow-up period of 86 months. The median recurrence free interval of these 26 patients was 7 months.

Comparing both studies. The Kaplan-Meier curve of the Proportion Recurrence-free Patients *versus* Months from TUR (Figure 1), clearly shows that the therapeutic effects after incomplete TUR are much better than after complete TUR.

Table III. *Patients' characteristics in Vilnius*.*

Patient number	Age (Years)	Gender	Stage/ grade tumours	Number of preoperative year	Recurrence rate/
1	77	F	pTaG2	>5	1.7
2	75	M	pTaG2	>5	0.9
3	43	M	pTaG2	4	2.1
4	53	M	pTaG2	>5	0.3
5	56	M	pTaG1	2	3.4
6	44	M	pTaG2	>5	0
7	69	F	pTaG1	1	0
8	78	M	pTaG1	2	0.4
9	73	M	pTaG2	>5	0.5
10	47	F	pTaG2	1	0
11	73	M	pTaG1	1	7.9
12	57	M	pTaG2	>5	1.1
13	92	M	pTaG2	>5	0
14	82	M	pTaG2	3	5.7
15	57	M	pTaG2	5	0
16	77	M	pTaG2	>5	0
17	68	F	pTaG2	>5	0.6
18	50	M	pTaG1	1	3.7
19	49	M	pTaG1	1	0
20	66	M	pTaG2	>5	2.3
21	69	M	pTaG3	2	0
22	66	M	pTaG3	>5	0
23	76	M	pTaG3	>5	0.3
24	61	M	pTaG3	1	0
25	66	M	pT1G3	>5	1
26	46	F	pT1G3	1	6.1

*These patients received complete TUR and IL-2 instillations (data from 16, 17).

Table V summarizes the statistical data comparing both studies. The percentage of patients with recurrence-free survival during the follow-up period after incomplete TUR was 2.2-times higher (50% *versus* 23%). The median of the length of the recurrence-free survival time after incomplete TUR was >2.9-fold that after complete TUR (>20.5 *versus* 7 months). The hazard ratio combining risk and event in censored and uncensored data, was 0.53 (95% confidence interval 0.23-1.19). Thus, all data indicated much better therapeutic results with regard to recurrences after incomplete TUR and complete TUR.

The patients characteristics differ between both studies. Table VI compares the major differences between the patient populations of both studies. The preoperative number of tumours was a little bit lower in Krakow compared to Vilnius, 3.1 *versus* 3.5, respectively. The recurrence rate per year was 2.2 *versus* 1.5, respectively. The T-stage in the two trials differ: all cases in the Krakow study and 8% in the Vilnius study were T1. In Krakow 8/10 of the tumours were G1; in Vilnius 6/26 were G1 14/26 were G2 and 6/26 were G3. The patients in Krakow were approximately 10 years younger, *e.g.* 56 compared to 66 years old in Vilnius. The percentage of males was lower in Krakow, 60% compared to 80% in Vilnius.

Table IV. *Time (months) between treatment and recurrence after complete TUR and local IL-2*.*

Patient number	Months between treatment and recurrence	No recurrence during whole follow-up period (Months)
1	2	
2	3	
3	2	
4	16	
5	10	
6	1	
7		>87
8	3	
9	6	
10		>86
11	34	
12	2	
13	17	
14	3	
15	3	
16	14	
17	38	
18	4	
19	79	
20	7	
21	7	
22	14	
23	68	
24	14	
25	2	
26	2	

*Clinical study performed in Vilnius; preliminary data have been published (16, 17).

The underlying question is how do all these differences influence the therapeutic outcome of both studies. Sylvester *et al*. (2) have provided weight estimates for various factors on the risk of recurrences after surgery for bladder carcinoma. The measured parameter values (columns 2 and 3 of Table VI) are weighed using the Sylvester weight estimates (column 4 and 5). Column 4 and 5 show the average weight of the parameter values of column 2 and 3 on recurrence risk, calculated from for each patient based on individual characteristics. Column 6 shows the predicted RFS after incomplete TUR based on the comparison of data in column 4 and 5; also other data in the literature are taken in account. A difference of more than 0.5 is considered better or worse, depending of its direction; differences between 0.1 and 0.5 are considered 'slightly'; differences less than 0.1 are considered minimal. So, the result is an educated guess of the influence of the differences in parameter values in both trials on the RFS. The predicted effect of T- and G-stage seem to balance each other between both studies. Sex and marker tumour are not reported to have significant effects. The small difference in preoperative tumours and the difference in age have only small opposite effects. The recurrence rate/year would indicate a worse prognosis for the Polish study. Taken together, the influence of the first seven parameters on the recurrence-free survival seems worse for the Polish study compared to the Lithuanian study, the weights for recurrence are 8.4 and 7.7, respectively. This means that after incomplete TUR more recurrences can be expected ; weight 8.4. This cannot explain the obviously opposite effect we found, as shown in Table V.

Discussion

IL-2 is thought to stimulate immune reactivity against tumour associated antigens (TAA). In other words, TAA should initiate an immune reaction, and IL-2 can stimulate this reaction. In line with this view, a recent review showed that local application of immunotherapeutics at the tumour site is much more effective than systemic application (7). However, after a well-performed TUR for NMIBC, no TAAs are left and consequently IL-2 cannot stimulate the immune reaction induced by TAA. This view is supported by the findings for patients 1 and 2 in Krakow: these patients were treated for recurrent bladder carcinoma with complete TUR for many years, but finally they were 'cured' after treatment with the antigenic marker tumour in combination with IL-2. The presence or absence of TAA could explain the therapeutic differences observed in these two pilot studies: taken together these data suggest that the presence of a marker tumour leads to improved therapeutic effects.

There are some differences between these two studies that have to be highlighted: Firstly, the difference in results may, partly, be due to differences in the technique of the TUR performed by individual surgeons (2). Secondly, the patients' characteristics differed between both groups (Table VI). These differences in patient characteristics seem to predict more recurrences after incomplete TUR than after complete TUR. However after therapy the opposite effect is obtained.

Unfortunately, no pathology review was part of these pilot studies. Thirdly, in Krakow IL-2 was dissolved in a solution of saline with 0.1% HSA, whereas HSA was not used in Vilnius. HSA was used in Krakow because IL-2 binds to HSA; this prevents IL-2 sticking to the wall of syringes, catheters *etc*. In Vilnius HSA was omitted; the IL-2 was dissolved in a relatively small volume of saline; this prevents precipitation of IL-2. Fourthly, in Krakow, 3×10^6 IU IL-2 were instilled daily, whereas in Vilnius 9×10^6 IU were instilled daily. Urologists in Vilnius preferred this higher dose as they expected better therapeutic results. However, a literature review showed that there is no linear relation between IL-2 dose and therapeutic effect (6). Moreover, a negative dose-response effect would be quite unexpected.

In both pilot studies, there were no side-effects observed due to intravesical IL-2 instillations.

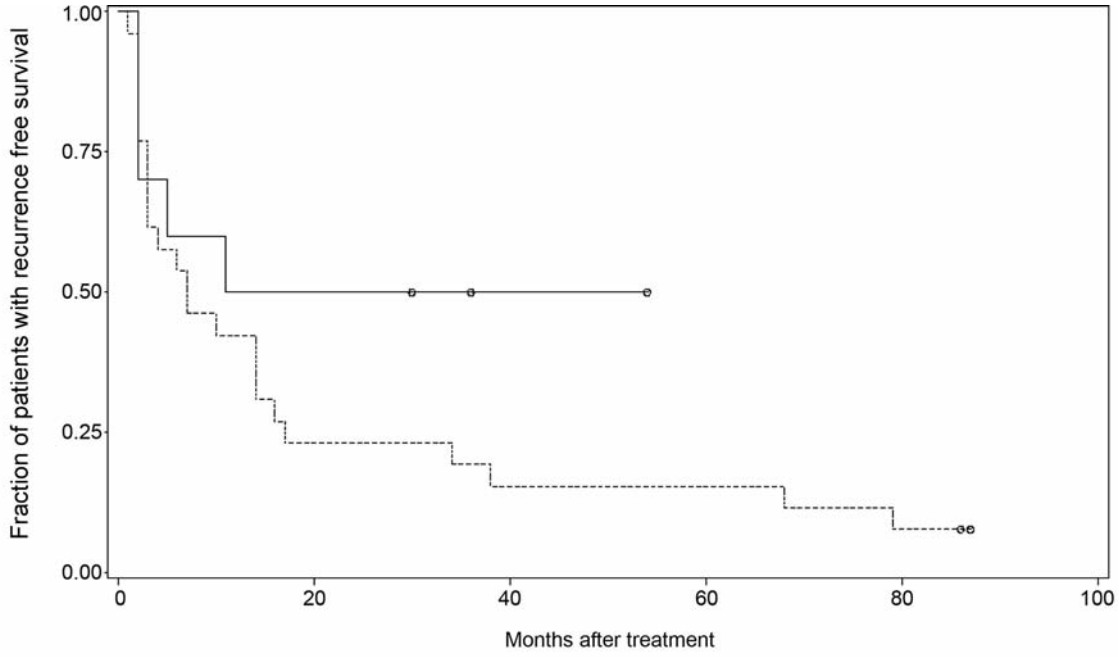

Figure 1. *Kaplan-Meier curve of the proportion of recurrence-free patients versus time after incomplete and complete TUR. The fraction of patients that were recurrence-free after local IL-2 and incomplete TUR (line; Krakow, Poland) and complete TUR (stripes; Vilnius, Lithuania). The X-axis indicates months after treatment with (in)complete TUR and local IL-2; the Y-axis indicates the fraction of patients having recurrence free survival. Circles are used to indicate censored data. Table V provides statistical analyses. Table VI summarizes differences in the patients populations of both studies and the predicted effect of several parameters values on the predicted RFS after incomplete versus complete TUR.*

Table V. *Therapeutic outcome of after incomplete and complete TUR and local IL-2 application.*

	Incomplete TUR	Complete TUR	Incomplete/ complete TUR	Therapeutic result incomplete TUR[b]
30 Months recurrence-free	50%	23%	2.2	Much better
Median recurrence-free survival (months)	>20.5	7	>2.9	Much better
Hazard ratio (Kaplan Meier)	[a]n.a.	n.a.	0.53	Much better

[a]n.a. Not applicable; [b]interpretation of therapeutic outcome from a patient's perspective

Beside the study in Krakow, seven other studies (11, 18-25) suggest that IL-2 instillation in the bladder may exert therapeutic effects. These data are summarized in Table VII. This table shows that direct contact between bladder carcinoma and IL-2 (column 2) leads to an impressive number of complete remissions (CRs) (column 3). In seven studies (that is all mentioned studies except that of Grasso *et al*, (24)) comprising 66 patients, there were 27 CRs (41%) and 8 partial remissions (PRs) (12%). That is very high, but similar to the proportion of patients without a recurrence during the follow-up that we found in Krakow. Although the number of patients is small, it is clear from the data in Table VII that contact between IL-2 and tumour cells can induce a CR. In the study of Grasso *et al*. (24) no CR was observed in 27 patients, but the percentage of historical recurrences per year was 95% and this was reduced to 33% in this study. Taken together, each of these eight studies indicates a clear therapeutic effect of local IL-2 in direct contact with bladder carcinoma. Only, the study of IL-2 installation after complete TUR of bladder carcinoma failed to show therapeutic efficacy.

We are aware that IL-2 is regarded as a stimulator of an effective cytotoxic reaction, as well as stimulating T-reg cell expansion leading to immune suppression (7, 25, 26). These seemingly contradictory effects of IL-2 may be due to differences in the doses of IL-2 that are used, the site of IL-2 application (7, 27), or the stage of the immune reaction, such as stimulation at an early or a late stage in the immune reaction. We did not observe any example of IL-2-induced stimulation of tumour growth.

Table VI. *Predicted outcome based on pre-treatment patient values.*

	Incomplete TUR	Complete TUR	Average weight incomplete TUR[a]	Average weight complete TUR[a]	Predicted RFS for incomplete TUR[b]
Preoperative number of tumours	3.1	3.5	3.6	3.7	Slightly better (2,16)
Recurrence rate/year	2.2	1.5	2.6	1.9	Worse (2,16)
T-stage	0% pTa 100% T1	92% pTa 8% T1	1	0.1	Worse (2)
G-stage	80% G1 20% G2 0% G3	23% G1 44% G2 23% G3	1.2	2.0	Better (2)
Mean age (years)	56	66	n.d.[d]	n.d.	Slightly worse (16)
% male	60%	81%	n.a.[e]	n.a.	No effect[c]
Tumour load during therapy	marker	no			Minimal (4, 5)
All factors combined			8.4	7.7	W0orse[f]

[a]Average weight in estimating risk of recurrence for patients that received incomplete (column 4) or complete TUR (column 5). Calculation performed for each patient was based on Sylvester *et al*. (2). The average values per group are displayed. [b]Predicted effect of differences between both studies (column 4 versus 5) on the recurrence-free survival after incomplete versus complete TUR. Estimation was mainly based on data in the literature (2, 4, 5, 16). Example: If literature states that less preoperative tumours lead to a better prognosis, and the patients with incomplete TUR have less preoperative tumours, then the predicted RFS is better. An average difference of more than 0.5 is considered better or worse, depending on its direction; differences between 0.1 and 0.5 are considered 'slight'; differences less than 0.1 are considered minimal. [c]DC, VP, JJLJ *et al*. unpublished data. [d]n.d., Not done; [e]n.a., not applicable; [f]mainly based on previous two columns.

Table VII. *Therapeutic effects of direct contact of IL-2 and carcinoma of the bladder.*

Reference	Contact of IL-2 with bladder carcinoma	Therapeutic effect	Recurrences
Den Otter *et al*. (6)	Marker lesion; intravesical IL-2 instillation	8/10 CR of marker tumor	4/8 recurrence
Pizza *et al*. (18)	Intralesional IL-2	3/6 CR; 2/6 PR; 1/6 massive necrosis	No recurrence during 2-7 months
Huland *et al*. (19)	Marker lesion; IL-2 perfusion	1/5 CR	No recurrence during 5 months
Gomella *et al*. (20)	Marker lesion; intravesical IL-2 instillation	3/14 CR; 1/14 PR	No recurrence during 3, 9, 9+ months
Ferlazzo *et al*. (21)	TUR + IL-2 perfusion of the bladder	8/9 CR	3/9 relapsed
Tubaro *et al*. (22)	Intra-arterial IL-2 infusion	2/12/ CR; 3/12 PR	
Velotti *et al*. (23)	Intra-arterial IL-2 infusion	2/10 CR; 2/10 PR	
Grasso *et al*. (24)	IL-2 instillation; tumors present	0/27 regression	Recurrence 9/27=33%. Historical controls 95%

CR, Complete regression; PR, partial regression: tumour size 1-50% of the original tumour.

The Kuhnian paradigm of immunotherapy of cancer is that an antigen, in this case TAA, induces a local immune reaction that leads to a cure (7). If a tumour is removed by a complete TUR, then TAA are not present to induce an immune reaction. The results described in this paper are exactly what the immunotherapeutic paradigm predicts.

It is clear that the therapeutic differences described in this article warrant a new study with the presence/absence of the marker tumour followed by IL-2 instillation. Currently we are performing such a randomized trial in the Netherlands (EudraCT number 2010-020397-42). If the data reported here are confirmed in this new ongoing trial, then the treatment of NMIBC with immunotherapy might be improved.

Conflicts of Interest

None.

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References

1 Brausi M, Collette L, Kurth K, Van der Meijden APM, Oosterlinck W, Witjes JA, Newling D, Bouffioux C, Sylvester RJ, EORTC Genito-Urinary Tract Cancer Collaborative Group: Variability in the recurrence rate at first follow-up cytoscopy after TUR in stage Ta-T1 transitional cell carcinoma of the bladder: A combined analysis of seven EORTC studies. Eur Urol *41*: 523-531, 2002.

2 Sylvester RJ, Van der Meijden AP, Oosterlinck W, Witjes JA, Bouffioux C, Denis L, Newling DW and Kurth K: Predicting recurrence and progression in individual patients with stage TaT1 bladder cancer using EORTC risk tables: A combined analysis of 2596 patients from seven EORTC trials. Eur Urol *49*: 466-477, 2006.

3 Lamm DL, Van der Meijden APM, Akaza H, Brendler C, Hedlund PO, Mizutani Y, Ratliff TL, Robinson MR and Shinka T : Intravesical chemo- and immunotherapy: How do we assess their effectiveness and what are their limitations and uses? Proceedings of the Fourth International Bladder Cancer Consensus Conference. Int J Urol *2(S2)*: 23-35, 1995.
4 Van der Meijden APM, Hall RR, Kurth KH, Bouffioux C, Sylvester R : Phase II trials in Ta, T1 bladder cancer. The marker tumour concept. Br J Urol *77*: 634-637, 1996.
5 Van der Meijden APM: The use of the marker tumor concept in TaT1 bladder cancer: Is it justified? Urol Oncol *7*: 31-33, 2002.
6 Den Otter W, Jacobs JJL, Battermann JJ, Hordijk GJ, Krastev Z, Moiseeva EV, Stewart RJE, Ziekman PGPM and Koten JW: Local therapy of cancer with free IL-2. Cancer Immunol Immunother *57*: 931-950, 2008.
7 Jacobs JJL, Characiejus D, Tomova R, Baran J, Bubenik J, Zembala M, Krastev Z, Scheper RJ, Pawelec G and Den Otter W: Local rather than systemic immunotherapy has therapeutic efficacy against metastasized cancer. Trends in Cancer Research *7*: 1-14, 2011.
8 Van Es RJ, Baselmans AH, Koten JW, Van Dijk JE, Koole R and Den Otter W Perilesional IL-2 treatment of a VX2 head and neck cancer model can induce a systemic antitumour activity. Anticancer Res *20*: 4163-4170, 2000.
9 Den Otter W, Hill FWG, Klein WR, Koten JW, Steerenberg PA, De Mulder PH, Rutten VP and Ruitenberg EJ: Low doses of interleukin-2 can cure large bovine ocular squamous cell carcinoma. Anticancer Res *13*: 2453-2455, 1993.
10 Stewart RJE, Hill FWG, Masztalerz A, Jacobs JJL, Koten JW and Den Otter W: Treatment of ocular squamous cell carcinomas in cattle using interleukin-2. Vet Rec *159*: 668-672, 2006.
11 Den Otter W, Dobrowolski Z, Bugajski A, Papla B, Van der Meijden AP, Koten JW, Boon TA, Siedlar M and Zembala M: Intravesical Interleukin-2 in T1 bladder carcinoma: regression of marker lesion in 8 out of 10 patients. J Urol *159*: 1183-1186, 1998.
12 Jacobs JJL, Hordijk GJ, Jürgenliemk-Schulz IM, Terhaard CHJ, Koten JW, Battermann JJ and Den Otter W: Treatment of stage III-IV nasopharyngeal carcinomas by external beam irradiation and local low doses of IL-2. Cancer Immunol Immunother *54*: 792-798, 2005.
13 Magno C, Melloni D, Gali A, Mucciardi G, Nicocia G, Morandi B, Melioli G and Ferlazzo G: The antitumor activity of bacillus Calmette-Guerin in bladder carcinoma is associated with an increase in the circulating level of interleukin-2. Immunol Lett *81*: 235-238, 2002.
14 Watanabe E, Matsuyama H, Matsuda K, Ohmi C, Tei Y, Yoshihiro S, OhmotoY and Naito K: Urinary interleukin-2 may predict clinical outcome of intravesical bacillus Calmette-Guerin immunotherapy for carcinoma *in situ* of the bladder. Cancer Immunol Immunother *52*: 481-486, 2003.
15 De Reijke TM, De Boer EC, Kurth KH and Schamhart DH: Urinary interleukin-2 monitoring during prolonged bacillus Calmette-Guerin treatment: Can it predict the optimal number of instillations? J Urol *161*: 67-71, 1999.
16 Characiejus D, Pašukonienė V, Jacobs JJL, Eidukevičius R, Jankevičius F, Dobrovolskienė N, Mauricas M, Van Moorselaar RJA and Den Otter W: Prognostic significance of peripheral blood CD8highCD57+ lymphocytes in bladder carcinoma patients after intravesical IL-2. Anticancer Res *31*: 699-703, 2011.
17 Jacobs JJL, Characiejus D, Pašukonienė V, Jankevičius F, Van Moorselaar RJA, Mauricas M and Den Otter W: Recurrences of superficial bladder carcinoma are associated with a raise of CD8highCD57+ and CD8low T lymphocytes in peripheral blood. Open Cancer Immunol J *3*: 8-14, 2010.
18 Pizza G, Severini G, Menniti D, De Vinci C and Corrado F: Tumour regression after intralesional injection of interleukin 2 (IL-2) in bladder cancer. Preliminary report. Int J Cancer *34*: 359-367, 1984.
19 Huland E and Huland H: Local continuous high dose interleukin 2: A new therapeutic model for the treatment of advanced bladder carcinoma. Cancer Res *49*: 5469-5474, 1989.
20 Gomella LG, McGinnis DE, Lattime EC, Butler K, Baltish M, Thompson I and Marshall ME: Treatment of transitional cell carcinoma of the bladder with intravesical interleukin-2: A pilot study. Cancer Biother *8*: 223-227, 1993.
21 Ferlazzo G, Magno C, Iemmo R, Rizzo M, Lupo G, Semino C, Bruno S and Melioli G: Treatment of superficial bladder cancer with intravesical perfusion of rIL-2: A follow-up study. Anticancer Res *16*: 979-980, 1996.
22 Tubaro A, Vicentini C, Bossola PC, Galassi P, Miano L, Velotti F, Santoni A, Pettinato A, Morrone S, Napolitano T, Frati L, Stoppacciaro A, Ruco L, Franks CR, Palmer PA and Pourreau CN: Continuous intra-arterial administration of recombinant interleukin-2 in low-stage bladder cancer. A phase IB study. Cancer *68*: 56-61, 1991.
23 Velotti F, Piccoli M, Frati L, Franks CR, Palmer P, Pourreau CN and Santoni A: Local/regional recombinant interleukin 2 (rIL-2) immunotherapy of tumors. Intra-arterial continuous infusion of rIL-2 in bladder cancer patients: a phase I study. Ann Ist Super Sanita *26*: 411-421, 1990.
24 Grasso M, Torelli F, Scannapieco G, Franzoso F and Lania C: Neoadiuvant treatment with intravesical interleukin-2 for recurrent superficial transitional bladder carcinoma Ta-T1/G1-2. J Immunother *24*: 184-187, 2001.
25 Sadoun D, Rosenzwaig M, Jily F, Six A, Carrat F, Thibault V, Sene D, Cacoub P and Klatzman D: Regulatory T-cell responses to low-dose interleukin-2 in HCV induced vasculitis. N Engl J Med *365*: 2067-2077, 2011.
26 Koreth J, Matsuoka K, Kim HT, McDonough SM, Bindra B, Alyea EP 3rd, Armand P, Cutler C, Ho VT, Treister NS, Bienfang DC, Prasad S, Tzachanis D, Joyce RM, Avigan DE, Antin JH, Ritz J and Soiffer RJ: Interleukin-2 and regulatory T cells in graft-*versus*-host disease. N Engl J Med *365*: 2055-2066, 2011.
27 Tomova R, Pomakov J, Jacobs JJL, Adjarov D, Popova S, Altankova I, Den Otter W and Krastev Z: Changes in cytokine profile during local IL-2 therapy in cancer patients. Anticancer Res *26*: 2037-2047, 2006.

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